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Dynamin: A Molecular Motor with Pinchase Action

Minireview

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The large GTPase dynamin, originally isolated from calf brain as a putative microtubule-associated motor enzyme nearly a decade ago (Shpetner and Vallee, 1989), has recently been implicated in the liberation of endocytic vesicles from the plasma membrane. A culmination of studies using either the temperature-sensitive mutant fruit fly *shibire*^{ts}, which possesses a point mutation near the GTP-binding domain of dynamin, or overexpression of mutant dynamin in mammalian cells is consistent with the concept that dynamin participates in clathrin-based endocytic processes. From these observations we had originally proposed that dynamin may act as a "molecular pinchase" (Urrutia et al., 1997) that could generate a mechanochemical force used to sever membranes. Appropriately, this concept has been challenged (Roos and Kelly, 1997). As recently reported by Sweitzer and Hinshaw (1998) and Takei and coworkers (1998), dynamin alone is capable of transforming spherical liposomes into long constricted tubules. Upon addition of GTP, dynamin subsequently severs these tubules into discrete vesicles of a consistent diameter (Sweitzer and Hinshaw, 1998). These studies make two substantial contributions. First, dynamin appears to meet the basic criteria of a molecular motor. Whereas the in vivo interactions between dynamin and cytoskeletal filaments are currently unresolved, it is now known that dynamin can bind and hydrolyze nucleotide to generate a motive force and do work, which, in this case, represents the compression and severing of a membrane tubule. Second, although it was thought that dynamin acted subsequently to the recruitment of coat proteins at the site of vesicle budding, both of these studies suggest that, at least under in vitro conditions, this is not the case.

Constricting Membranes into Tubules

The dynamin family of proteins is encoded by three distinct genes and has several conserved motifs (Figure 1) that provide insight into its function. These include a highly conserved tripartite GTP-binding domain in the first N-terminal 300 amino acids; a pleckstrin homology domain (PHD) of 100 amino acids, which may mediate membrane binding; and a proline-rich domain (PRD) at the C terminus, which is modestly conserved and believed to mediate interactions between dynamin and other proteins (reviewed by Urrutia et al. 1997).

The first mechanistic insights into how dynamin might interact with either itself or membranes to form invaginations was provided by seminal studies using cell-free systems. Tuma and Collins (1994) were the first to demonstrate that the GTPase activity of dynamin exhibits positive cooperativity with regard to enzyme concentration, and that direct interactions between dynamin molecules may regulate function. Subsequently, Hinshaw and Schmid (1995) provided graphic images of dynamin, under low salt conditions, assembling into rings and spirals. These structures were of similar dimensions to the "collars" about the membrane invaginations observed at the nerve terminals of mutant *shibire*^{ts} flies (Kosaka and Ikeda, 1983; Koenig and Ikeda, 1996). This self association of dynamin was shown not to require GTP binding or hydrolysis. Thus, dynamin is the second structural protein, after clathrin, known to undergo self assembly to mediate coated vesicle formation. At nearly the same time Tuma and Collins (1995) demonstrated that dynamin has an intrinsic capacity to form polymeric complexes along lipid vesicles. Together these groups provided the first predictions of how dynamin polymerization could facilitate the fission of an endocytic membrane tubule from the cell surface. Takei and coworkers (1995) provided the first demonstration that membranes from hypotonically lysed rat brain synaptosomes, incubated with brain cytosol and GTPγS, could support the formation of tubular invaginations decorated with dense staining rings of dynamin. The physical similarity between these dynamin-coated membrane structures and those found either in the mutant *shibire*^{ts} nerve terminals in vivo or in the cell-free studies of dynamin spirals (Hinshaw and Schmid, 1995), could not have been more striking.

Despite the initial appeal of the important observations described above, additional experimentation was required to confirm their physiological relevance. First, did the collared invaginations formed in the lysed synaptosomal preparations represent an ungoverned assembly of dynamin induced by GTPγS? Second, why were low salt conditions ($<$50 mM) required for the cell-free formation of polymeric dynamin ring structures? Finally, can dynamin sever membranes alone, or does it play a secondary function as a cofactor or a "molecular switch" characteristic of most GTPases? The first of these questions is addressed by Takei and coworkers (1998) who have extended their original findings using rat synaptosomal membrane to include membranes and lipids from several cellular and synthetic sources. The modifications included the use of a highly enriched preparation of dynamin-deficient coat proteins stripped from brain-purified clathrin-coated vesicles and/or purified

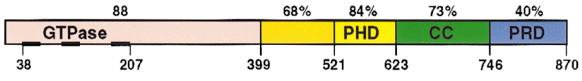

Figure 1. The Dynamin Proteins Possess Specific Domains That Perform Distinct Functions

Illustration depicting percent homology between the different domains of dynamin 1, 2, and 3. A highly conserved tripartite GTP-binding domain is located at the N terminus, while a membrane-binding, pleckstrin homology domain (PHD) is more centrally situated. A region, weakly predicted to be a coiled-coil (cc), may act as an effector domain to activate the dynamin GTPase activity and lies just before a proline-rich domain (PRD) that has been demonstrated to interact with numerous other proteins. This figure does not represent the dynamin-related proteins that do not have a PHD or a PRD.

recombinant dynamin 1. These protein components were combined with lipid membranes under a variety of conditions, most notably, either ATP with GTP, or ATP with GTPγS. From these variations of a central theme several surprising and important observations were made. First, the membrane requirements for dynamin to make constricted multicollared tubules is not as specific as was originally predicted. Although dynamin did not associate with mitochondrial or nuclear membranes, collared structures formed, not only on membranes of perforated CHO cells in culture, but also along inside-out red blood cell membranes and even on protein-free liposomes. Second, in addition to participating in the incorporation of internalized cargo and contributing to both curvature and constriction of a forming tubular vesicle, coat proteins may also act as governors to either facilitate or limit the association of dynamin and other proteins with an organelle surface. Thus, an additional function for clathrin might be to provide a structural barrier to prevent the rapid and unchecked polymerization of dynamin onto a forming tubule. Perhaps most importantly, the recent study by Takei and coworkers (1998) demonstrates that collared tubules can form in vitro without the nonhydrolyzable analog GTPγS or even ATP or GTP. Although GTPγS clearly enhances the visualization of dynamin-collared tubules, such structures clearly form under other conditions. Thus, nucleotide is not required for either dynamin-coat recruitment to membranes or subsequent tubule formation.

Pinching Tubules into Vesicles

The findings of Takei and coworkers (1998) are complementary to the observations of Sweitzer and Hinshaw (1998), which established dynamin's "pinchase" function. Although the experimental conditions in the Takei study supported the formation of long dynamin-coated tubules, with or without clathrin, liberation of individual vesicles did not occur under any condition tested. Steps toward defining the conditions for optimal dynamin function were achieved by a recent study (Carr and Hinshaw, 1997) in which addition of GDP and γ-phosphate analogs promoted the assembly of recombinant dynamin 1 into long spirals under physiological salt concentrations. In the current study (Sweitzer and Hinshaw, 1998) these conditions were refined further so that dynamin 1, when combined with synthetic liposomes composed of phosphatidylserine, formed a regular pattern of helical coated membranes, which not only constricted spherical liposomes into tubules but vesiculated these tubules upon addition of GTP. Remarkably, dynamin alone can bind, constrict, and sever membranes into individual vesicles. Whereas the first two steps of this process can occur in a nucleotide-independent manner, the final fission step requires GTP. This observation is consistent with the early observations made both in the mutant *shibire*^{ts} flies (Koenig and Ikeda, 1996) and in the in vitro studies using GTPγS (Takei et al., 1995). Either because of a point mutation in or near the GTP-binding motif of the fly dynamin or the presence of the GTPγS analog, GTP hydrolysis is prevented, thereby facilitating the accumulation of yet more collared invaginations. Subsequent liberation of nascent vesicles cannot occur until dynamin hydrolyzes GTP. In addition to this striking observation, Sweitzer and Hinshaw (1998) demonstrate that dynamin can bind, constrict, and sever membrane tubules into vesicles even after the proteolytic removal of the C-terminal PRD. This informative experiment is consistent with a model in which the PHD and N-terminal portions of dynamin provide membrane and nucleotide binding as well as a pinchase function. The C-terminal PRD may protrude outward from the membrane tubule to bind targeting proteins, such as SH3 domain–containing proteins or the membranes of specific organelles.

Correlating the Action of Dynamin In Vitro with Vesicle Formation in Living Cells

From the cell-free studies described above we have gained additional insights into how dynamin and coat proteins interact to form membrane tubules and vesicles. Are there parallels between the in vitro observations and vesicle biogenesis in an intact cell? One is inclined to say yes based on the comparative images of vesicle formation in vivo and in vitro shown in Figure 2. This figure shows similar images of membrane tubules observed in intact neurons and epithelial cells in which dynamin function has, or has not, been impaired. The common theme conveyed by these images is that these complex and transient tubular networks may normally reside at the cell cortex but are accentuated when dynamin function is disrupted, either by antibodies or a temperature-sensitive point mutation. It is attractive to predict that these dynamin-and coat-laden reticular membrane tubules provide a template from which nascent vesicles are generated. Rather than a single coated vesicle assembling and releasing from a donor membrane, compartments may form tubular reticuli, which are subsequently constricted by coat proteins into segments or links. Activation of dynamin would provide a terminal scission event transforming the tubule into multiple vesicles. This tubule model for vesicle biogenesis has been proposed to occur at the *trans*-Golgi network (Rambourg et al., 1981).

The models described here are perhaps best supported by the elegant studies of Koenig and Ikeda (1996) who have examined the formation and vesiculation of tubular membrane intermediates in retinula cell terminals from *shibire*^{ts} flies. When these flies are held for 30–60 s at the restrictive temperature followed by a 5 min recovery at the permissive temperature, constricted membrane tubules are formed in these cells (Figure 2d). As the recovery period is extended, these tubules become increasingly constricted until the reticulum is consumed and transformed into a cluster of vesicles (Figures 2e and 2f). This vesiculation of a reticular intermediate, which occurs away from the active zone at the terminal, is believed to be slower than the tubule-to-vesicle transition at the active zone, which may occur too quickly to capture using conventional chemical fixation methods. Thus, it is attractive to predict that this process in the intact *shibire*^{ts} cells is represented by the constriction of tubules observed in the cell-free assays. How this mechanism could produce vesicles of a consistent size without the participation of clathrin coats is unclear.

An additional parallel between tubule-vesicle formation in vitro and in *shibire*^{ts} nerve terminals is the apparent lack of a clathrin requirement. Clathrin coats may aid vesicle formation in vitro by budding membrane and providing a curvature of consistent dimensions. The most striking observation, however, is that nascent synaptic vesicles at retinula cell terminals of *shibire*^{ts}, either

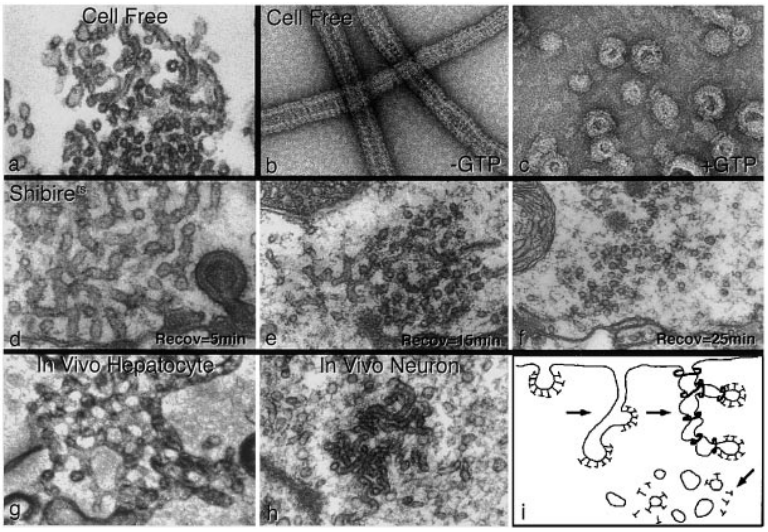

Figure 2. Dynamin Mediates the Formation of Tubular-Vesicular Complexes in Cells or in Cell-Free Assays

A gallery of electron micrographs collected from studies demonstrating a role for dynamin in the formation of membrane reticuli and discrete vesicles.

(a–c) Formation of tubular-vesicular complexes in vitro. (a) Liposomes comprised of brain lipids form a tubular reticulum in the presence of brain cytosol, ATP, and GTP. (b) Recombinant dynamin 1 is assembled into spiral structures, which constrict phosphotidyl serine liposomes into extended tubules of uniform diameter in the absence of GTP. (c) Addition of 1 mM GTP to the dynamin-liposome tubule preparation depicted in (b) induces vesiculation.

(d–g) Disruption of dynamin function in living cells induces membrane morphologies similar to those observed in vitro.

(d–f) Membrane dynamics in retinula nerve cell terminals from the *shibire^{ts}* mutant flies during recovery from the restrictive temperature. (d) A 5 min recovery allows the formation of numerous branching reticular tubules. (e) By 15 min, clumps of markedly vesiculated tubules form. (f) These membrane tubule clumps resolve into discrete vesicles by 25 min of recovery. This process appears to mimic the in vitro vesiculation of liposomes by dynamin shown in (c).

(g) Inhibition of dynamin function in a cultured hepatocyte microinjected with purified dynamin antibodies. Several hours after injection, reticular plasma membrane invaginations, resembling those observed in vitro and in the *shibire^{ts}* neurons, are formed.

(h) Untreated mammalian photoreceptor nerve terminals in situ reveal a tubular reticulum.

(i) A model, incorporating the morphological observations shown above, predicts that newly formed membrane buds, coated or noncoated, grow and extend from a donor compartment. The assembly of dynamin on these tubules may generate a membrane reticulum from which nascent vesicles bud.

(a) Reproduced with permission from Takei et al. (copyright 1998, Cell Press). (b and c) Reproduced with permission from Sweitzer and Hinshaw (copyright 1998, Cell Press). (d, e, and f) Reproduced with permission from Koenig and Ikeda (Rockefeller University Press, 1996). (g) Reproduced with permission as modified from Henley et al. (Rockefeller University Press, 1998). (h) Courtesy of Takei and De Camilli.

at or away from the active zone, are not clathrin-coated. This lack of clathrin coats is likely to be biologically significant because many aberrant clathrin-coated pits are observed in epithelial cells from the same mutant flies (Kosaka and Ikeda, 1983). This suggests that coat components in the neurons are not lost due to the fixation process, and that formation of at least some vesicular populations at the synapse is clathrin-independent.

Lipids, Coats, and Dynamin

Although it is generally assumed that dynamin and clathrin work together to form nascent vesicles from the plasma membrane, there are several examples in which vesicles may be generated without either protein. Matsuoka et al. (1998) have identified the components of the COPII complex that are competent to form vesicles from purified liposomes in a defined assay. The COPII coat has been implicated in the formation of nascent vesicles from the endoplasmic reticulum. Remarkably, the combination of only three COPII components, Sar1p, Sec13/31p, and Sec23/24p, with phosphoinositol 4-phosphate and specific phospholipids supports the formation of coated buds and vesicles at both 30°C and 4°C. Thus, only five proteins appear to be required to generate lipid vesicles in vitro without the aid of dynamin. To reduce the requirements of vesicle formation further, Zha et al. (1998) have recently demonstrated that cultured cells, depleted of ATP and incubated with exogenous sphingomyelinase, rapidly form numerous noncoated, ligand-containing vesicles that bud from the plasma membrane. These authors speculate that hydrolysis of sphingomyelin alone may cause inward curvature and subsequent membrane scission to form sealed endocytic vesicles. Although these observations do not obviate the requirement for any particular coat protein in vesicle biogenesis, they do indicate that cells may use several different mechanisms to form nascent vesicles.

Compressing, Slicing, or Dicing Membrane Tubules?

Our understanding of dynamin function is based on the initial biochemical observations made by Hinshaw and Schmid (1995) and Tuma and Collins (1994, 1995). From these findings dynamin action can be organized into at least three distinct processes: binding/assembly, severing, and disassembly.

During the binding/assembly reaction, a dynamin homodimer or homomultimer may be targeted to a specific membrane compartment through the binding of its PRD tail to the SH3 domain of a specific protein on the target membrane. Concomitant with, or subsequent to, this protein–protein binding is an association of the dynamin PHD with a negatively charged lipid surface. Upon membrane binding and without a nucleotide requirement, dynamin adopts an orientation that promotes self assembly into spirals (Figure 2b) which may act to constrict flat membrane surfaces into tubules. This binding and constriction process is likely to be enhanced or regulated substantially through the synergistic assembly of various coat proteins.

The severing reaction may occur as follows. As a result of self assembly the GTPase activity of dynamin is increased dramatically inducing a predicted conformational change in the dynamin polymer. Although this

active, nucleotide-dependent constriction may account for only a 20%–30% reduction in the circumference of a membrane tubule (Sweitzer and Hinshaw, 1998), it is possible that this change exceeds the curvature limit permitted for a stable lipid bilayer. Thus, the membrane may be snapped or broken in distinct locations rather than being cut.

Finally, following a conformational change in the dynamin polymer and the liberation of nascent vesicles, dynamin may release GDP and P_i to disassemble into smaller oligomers and detach from the membrane surface. Alternatively, dynamin may remain associated with a newly formed vesicle for transport to another target membrane.

Although simplified and incomplete, this scenario provides a model that can be tested in the future. The cell-free studies described here demonstrate that dynamin alone can compress and sever membrane tubules. It will be of interest to test how this reaction is governed in living cells. In particular, understanding the physical interactions between dynamin, an invaginating membrane tubule, and the cytoskeleton will prove particularly important. It is attractive to predict that either the microtubule- or actin-based cytoskeleton could participate in a dynamin-mediated vesicle budding event by pulling on a forming membrane tubule. Such a force would place the invaginating membrane under tension, thereby facilitating a dynamin-based compression and severing. Although vesicle formation in cells is generally believed not to be directly dependent upon microtubules, it is possible that such interactions may enhance the efficiency of this process. Furthermore, the actin-myosin cytoskeleton has been implicated in the formation of nascent secretory and endocytic vesicles from the *trans*-Golgi network (Müsch et al., 1997) and the plasma membrane (Salisbury et al., 1980; Munn et al., 1995), respectively. Thus, vesicle formation may proceed by the synergistic efforts of multiple force generating enzymes pulling, pushing, and pinching membranes simultaneously.

Future Directions

Although our understanding of how dynamin generates a compression force is currently embryonic, rapid progress should be made by utilizing the biochemical, molecular, and cell biological methods applied to other molecular motor enzymes. Whether dynamin performs other functions via its interaction with numerous signaling SH3 domain–containing proteins also needs to be defined. Finally, it will be important to understand how dynamin assembly and activity are regulated, and how the different dynamin proteins might be targeted to distinct membrane compartments. With the number of identified dynamin family members increasing, it is attractive to draw a parallel with other molecular motor families that are known to function at distinct membrane compartments. Indeed, dynamin has been shown to participate in the liberation of caveloae (Henley et al., 1998; Oh et al., 1998) and in vesicle trafficking to and from the Golgi apparatus (Jones et al., 1998; Llorente et al. 1998). How the activity of different dynamin molecules at distinct membrane compartments might be modulated is undefined. So, the dynamin founders were on target from the start: it appears to be a motor after all.

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